High plasma estradiol interacts with diabetes on risk of dementia in older postmenopausal women

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ABSTRACT

Objective: We aimed to investigate the impact of endogenous estradiol (E2) on dementia and to evaluate the contribution of vascular risk factors and inflammatory and blood coagulation markers to this association.

Methods: Using data from a French population-based prospective study (the Three-City Study) including 5,644 postmenopausal women aged 65 years or older, we investigated the association of endogenous total-E2 and bioavailable-E2 and total-testosterone with the 4-year incidence of all-cause dementia. We further focused on the role of dementia and cardiovascular risk factors as well as inflammation (C-reactive protein, fibrinogen) and hypercoagulability (fibrin D-dimers, thrombin generation) in these associations. We used a case-cohort design consisting of a random subcohort of 562 women not using hormone therapy and 132 incident dementia cases.

Results: Adjusted Cox proportional hazards models showed a J-shaped relationship between total-E2 and risk of dementia ($p = 0.001$). Total-E2 values in the lower and upper quartiles were associated with an increased dementia risk (adjusted hazard ratio [HR] [95% confidence interval] = 2.2 [1.1–4.5] and HR = 2.4 [1.2–5.2], respectively). Importantly, the risk associated with higher E2 levels was dramatically increased in women with diabetes compared with nondiabetic women (adjusted HR associated with the upper E2 quartile = 14.2 [1.60–123] and HR = 3.4 [0.1–147], respectively, p interaction <0.05). Similar results were found for bioavailable-E2. Adjustment for inflammatory and blood coagulation markers did not modify our results. No significant association was found for total-testosterone.

Conclusion: High E2 level is an independent predictor of incident dementia, particularly in postmenopausal women with diabetes. ***Neurology*® 2014;82:504–511**

GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; ***DSM-IV-R*** = *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, revised; **DVC** = dementia with vascular component; **E2** = estradiol; **GM** = geometric mean; **HDL-C** = high-density lipoprotein cholesterol; **HR** = hazard ratio; **hs-CRP** = high-sensitivity C-reactive protein; **SSH** = steroid sex hormone; **T** = testosterone; **WHR** = waist-to-hip ratio.

It has long been presumed that the menopause-related rapid decline of estradiol (E2) levels was involved in women's cognitive decline and that hormone therapy might be beneficial for the prevention of cognitive impairment and dementia. However, data from clinical trials mostly failed to show benefit of estrogen-based therapy,[1] and the Women's Health Initiative Memory Study even showed a higher dementia risk in older women using hormone therapy.[2]

Few prospective studies have investigated the relationship between endogenous E2 and dementia risk, while observational studies on cognitive decline led to inconsistent findings among postmenopausal women.[3–5] Two studies found an increased risk of vascular dementia[6] and Alzheimer disease (AD)[7] in women with high E2 levels. Although unexpected, these results are consistent with recent data supporting a deleterious effect of high E2 levels on frailty,[8] cardiovascular disease,[9] and all-cause mortality[10] among postmenopausal women.

Supplemental data at www.neurology.org

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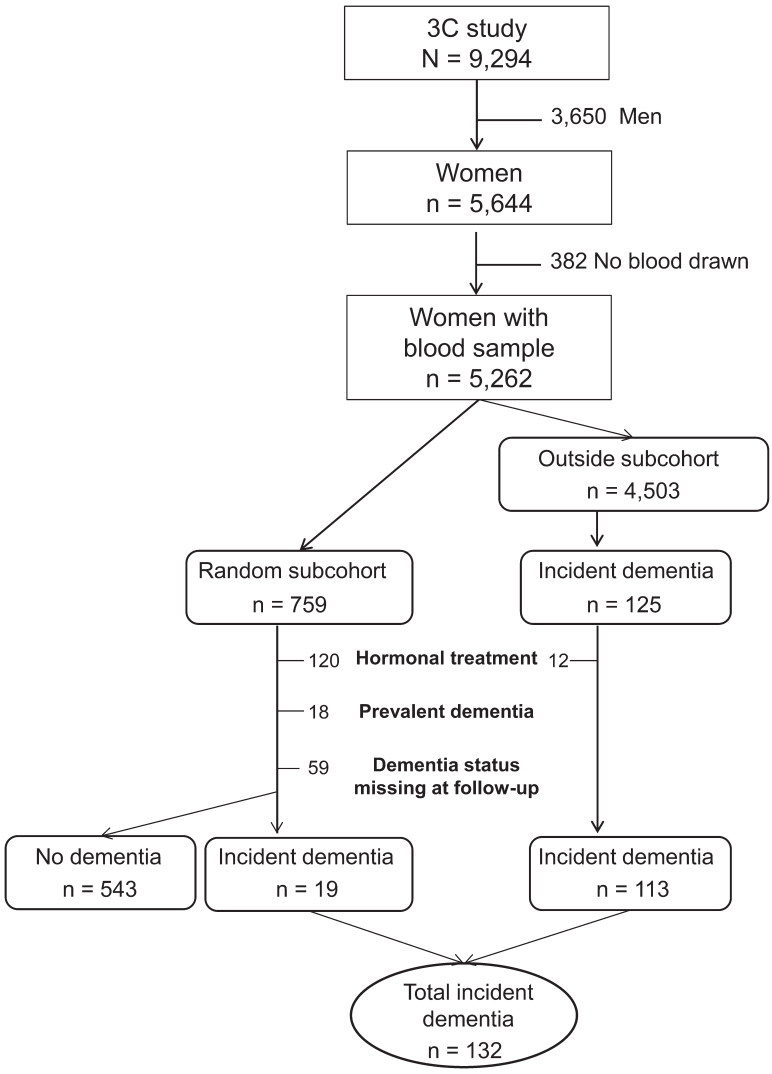
Figure 1 Sample selection



Flowchart of study sample selection. 3C = Three-City.

Higher endogenous E2 may have detrimental effects in the brain through adverse vascular mechanisms, including diabetes,[11] adverse lipid profile,[12] and enhanced inflammation and coagulation,[13,14] factors that may in turn be implicated in the dementia process.[15–18] Furthermore, the *APOE* gene, a determinant of cardiovascular disease and dementia, may influence the association between sex hormone and dementia.[19,20]

We aimed to investigate the association of total and bioavailable (Bio) endogenous E2 and total testosterone (T) on the 4-year dementia risk in older postmenopausal women from the Three-City Study and to evaluate the possible interaction of several vascular risk factors with E2 on this risk.

METHODS

Population study. The Three-City Study is an ongoing French prospective cohort study of vascular risk factors for dementia in the elderly. Details of the protocol have been described previously.[21] Briefly, 9,294 community dwellers (including 5,644 women) 65 years or older, selected from electoral rolls of 3 French cities (Bordeaux, Dijon, and Montpellier), were included between March 1999 and March 2001.

Subjects were then followed up every 2 years for the detection of cardiovascular disease and dementia. Data up to the second follow-up visit (4 years) are used in this study.

Standard protocol approvals, registrations, and patient consents. Each participant signed an informed consent and the study protocol was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre.

Case-cohort design. After the second follow-up evaluation, a case-cohort design was set up for biological investigations. In this design, a random sample of 759 women (subcohort) was selected from the population of women, within strata of center and age (figure 1). In addition, 125 cases of dementia detected outside the subcohort were added. After exclusion of women using hormonal treatment (n = 132), having prevalent dementia (n = 18), or having a missing dementia status at follow-up (n = 59), the final sample consisted of 543 noncases and 132 cases of incident dementia. Baseline characteristics of women included in the subcohort were compared with those from the remaining study sample and no significant difference was detected.

Diagnosis of dementia and neuropsychological assessment. A 2-step procedure was used for the detection of prevalent and incident dementia. After a neuropsychological examination, all subjects with suspected dementia based on their neuropsychological performances or decline since a previous assessment underwent further examination by a neurologist. All potential cases of dementia were then reviewed and ascertained by an independent committee of neurologists using *DSM-IV-R* criteria.[22] Etiology was defined according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for AD and National Institute of Neurological Disorders and Stroke–Association Internationale pour la Recherche et l'Enseignement en Neurosciences criteria for vascular dementia. In this study, mixed dementia (defined as AD with vascular lesions) and pure vascular dementia were combined to form a category of dementia with vascular component (DVC). Incident dementia status was available for 90% of the study population at 4 years of follow-up.

Cognitive impairment was defined as a score in global (evaluated using the Mini-Mental State Examination[23]), verbal (evaluated by Isaac Set Test[24]), or visual memory (evaluated by Benton Visual Retention Test[25]) in the lower 10th percentile of the age- and education-stratified tests' score distribution.

Laboratory measurements. At baseline, blood samples were collected for 90% of the entire cohort in the morning after an overnight fast. Centralized measures of fasting blood glucose, low- and high-density lipoprotein cholesterol (HDL-C), and triglycerides were performed. Details on steroid sex hormones (SSHs)[9,26] and coagulation markers[17,27] assessment have been described. Plasma total-E2 and total-T were measured using radioimmunoassay methods. Minimum detectable concentration of total-E2 and total-T were 2 pg/mL (7.3 pmol/L) and 0.02 ng/mL (0.06 nmol/L), respectively. Bio-E2 concentration was measured by an indirect method after differential precipitations of E2 bound to globulins with 50% ammonium sulfate and equilibration of the plasma sample with [^{3}H]-estradiol. High-sensitivity serum

C-reactive protein (hs-CRP) was measured using a particle-enhanced turbidimetric immunoassay.[28] Fibrinogen was measured using the Clauss method and fibrin D-dimer using ELISAs.[17] Thrombin generation was measured by the calibrated automated thrombography.[27,29] We used peak height (highest value of thrombin generated) and endogenous thrombin potential (total quantity of thrombin generated) in our analyses.

Covariate assessments. At baseline, trained nurses or psychologists conducted face-to-face interviews at home or at a study center using a standardized questionnaire. Information on sociodemographic characteristics (age, sex, and education), medication use, and lifestyle habits were registered. In addition, brachial blood pressure and height and weight measurements were collected during a physical examination.

Other covariates included hypertension (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, or if treated), hypercholesterolemia (total cholesterol ≥6.20 mmol/L^{-1} or if treated), diabetes (fasting blood glucose >7 mmol/L^{-1} or a treatment), self-reported history of coronary heart disease (including myocardial infarction, angina pectoris, vascular surgery) and stroke, *APO* ε4 genotype (performed at the French Lille Genopole, http://www.genopole-lille.fr), and depressive symptoms (defined as a score on the Center for Epidemiologic Studies Depression Scale ≥16).[30]

Statistical analysis. Except for age, for which a Student *t* test was used, comparisons of characteristics between cases and noncases were performed using Cox proportional hazards models with modification of the standard errors based on robust variance estimates as recommended for a case-cohort study design.[31] Because dementia is an age-related disease, age was used as the timescale in our models. Proportional hazards assumption was checked (and met) for all variables using log vs log minus log plot. Geometric means (GMs) and interquartile ranges for biological measures were log-transformed to better approach normal distribution. Age-adjusted partial correlations between SSHs and body mass index (BMI), waist-to-hip ratio (WHR), HDL-C, low-density lipoprotein cholesterol, triglycerides, glycemia, hs-CRP, fibrinogen, D-dimers, and thrombin generation were estimated. The possibility of linear or quadratic associations of SSHs with dementia was studied. Hazard ratios (HRs) associated with quartiles of SSHs (based on noncases' SSH distributions) are shown. Deviation from linearity was tested using appropriate log-likelihood ratio tests and inclusion of a quadratic term for SSHs was tested when the relationship actually deviated from linearity. The associations of SSHs with all-cause dementia, AD, and DVC, were adjusted for age and study center in a first model. A second model was further adjusted for education, Mini-Mental State Examination score, *APOE* ε4, WHR, depression, hypercholesterolemia, diabetes, and prevalent cardiovascular disease, in the absence of significant interaction between these factors and the hormone studied. Additionally, confounding and effect modification by biological parameters, including lipids, inflammation, and coagulation markers were assessed because they could be involved in the causal pathways linking SSHs to dementia. Because data were missing for some variables, particularly for WHR (11%), multiple imputations were used for missing data in the final models. Five imputed data sets were created and analyzed together and results were combined using Rubin's rules.[32]

Sensitivity analyses were performed to evaluate the robustness of our findings. Because we previously reported an association of E2 levels with cardiovascular disease in the same study,[9] we estimated the association of total-E2 and Bio-E2 with all-cause dementia excluding all prevalent and incident cases of cardiovascular disease. Furthermore, to limit the possibility of reverse causation, subjects presenting with cognitive impairment at baseline or developing dementia within the first 2 years of follow-up were excluded in a secondary analysis.

RESULTS

Women's characteristics at baseline. Overall, the study sample comprised 543 noncases and 132 dementia cases (figure 1). Older age ($p < 0.0001$), higher WHR ($p < 0.01$), lower education ($p = 0.01$), *APOE* ε4 ($p < 0.001$), diabetes ($p = 0.05$), and depressive symptoms ($p < 0.001$) were significantly associated with an increased risk of dementia (table 1). Moreover, women with dementia had higher baseline HDL-C ($p = 0.03$), triglycerides ($p = 0.01$), and D-dimer levels ($p = 0.04$). Total-E2 and Bio-E2 levels were not different according to dementia status ($p = 0.99$ and $p = 0.87$, respectively). Finally, total-E2 levels were significantly and positively correlated with BMI ($r = 0.26$), WHR ($r = 0.10$), hs-CRP ($r = 0.37$), fibrinogen ($r = 0.23$), triglycerides ($r = 0.17$), and glycemia ($r = 0.12$). Analysis of Bio-E2 led to similar results.

Risk of dementia associated with SSHs. There was a J-shaped relationship between total- or Bio-E2 levels and all-cause dementia ($p = 0.001$); both lower and higher levels of E2 were associated with dementia (table 2). After multivariate adjustment, values of E2 in the lower and higher quartiles were associated with dementia ($HR_{total\text{-}E2}$ [95% confidence interval] = 2.43 [1.15–5.20], $p = 0.02$, and $HR_{total\text{-}E2} = 2.20$ [1.07–4.52], $p = 0.03$, respectively). Similar associations were found when restricting the analysis to AD. The small number of DVC cases precluded possible statistical significance; results are displayed in table e-1 on the *Neurology*® Web site at www.neurology.org. Further adjustment for lipids, inflammation, or coagulation markers did not alter the relationship, and neither did adjustment for testosterone levels.

Interaction of vascular risk factors with E2 on dementia risk. Diabetes was the only factor found to modify the effect of E2 on dementia (p for interaction = 0.04 for total-E2 and $p = 0.01$ for Bio-E2). Mean variation of total- and Bio-E2 among women with and without dementia was very different according to diabetes status. In women with diabetes, total- and Bio-E2 levels were approximately 70% higher in women with dementia compared with women without dementia ($GM_{total\text{-}E2} = 9.0$ pg/mL vs $GM_{total\text{-}E2} = 5.6$ pg/mL, $p = 0.03$; $GM_{Bio\text{-}E2} = 6.3$ pg/mL vs $GM_{Bio\text{-}E2} = 3.7$ pg/mL, $p = 0.03$), while no significant difference was observed in nondiabetic women. Figure 2 shows the risk for all-cause dementia associated with total-E2 according to diabetes status. Among diabetic women, risk of dementia was dramatically increased in those with a value of total-E2 in the highest quartiles (multivariate-adjusted HR = 14.2 [1.60–123], $p = 0.02$ vs HR = 3.42 [0.08–147], $p = 0.02$ in nondiabetic women). In this subgroup, the association of E2 with dementia did not deviate from linearity ($p = 0.48$) and linear trend was borderline significant ($p = 0.05$).

Table 1 Women's characteristics at baseline according to all-cause dementia status

Characteristics	Noncases (n = 543)	All-cause dementia (n = 132)	p[a]
Age, y, mean (SD)	74.4 (5.3)	78.7 (5.0)	<0.0001
Center, n (%)			0.02
Bordeaux	117 (21.6)	49 (37.1)	
Dijon	297 (54.7)	57 (43.2)	
Montpellier	129 (23.8)	26 (19.7)	
BMI, kg/m^2, mean (SD)	25.6 (4.6)	24.8 (4.5)	0.26
WHR, mean (SD)	0.84 (0.07)	0.86 (0.06)	<0.01
Education, n (%)			0.01
Less than grade school	203 (37.4)	65 (49.2)	
Grade school or high school	168 (30.9)	28 (21.2)	
High school validated or university	172 (31.7)	39 (29.6)	
APOE ε4 carriers, n (%)	104 (19.2)	40 (30.3)	<0.001
Hypercholesterolemia,[b] n (%)	75 (19.0)	57 (20.4)	0.28
Hypertension,[c] n (%)	415 (76.4)	110 (83.9)	0.70
Diabetes,[d] n (%)	41 (7.8)	16 (12.1)	0.05
Smoking status, n (%)			0.16
Never	442 (81.4)	119 (90.2)	
Past	78 (14.4)	10 (7.6)	
Current	23 (4.2)	3 (2.3)	
Personal history of stroke, n (%)	22 (4.1)	8 (6.2)	0.30
Personal history of coronary disease, n (%)	48 (8.8)	26 (19.7)	0.03
Depressive symptoms,[e] n (%)	76 (14.2)	38 (29.2)	<0.001
Age at menopause, y, mean (SD)	49.3 (5.87)	48.5 (5.5)	0.12
MMSE score at baseline	27.3 (2.0)	25.7 (2.3)	<0.0001
Laboratory parameters, GM (IQR)			
Total-E2, pg/mL	5.31 (3.45–8.00)	5.26 (2.75–9.30)	0.99
Bio-E2, pg/mL	3.53 (2.29–5.58)	3.46 (1.68–6.23)	0.87
Total-T, ng/mL	0.30 (0.21–0.45)	0.28 (0.20–0.46)	0.31
HDL-C, mmol/L	1.68 (1.46–1.96)	1.59 (1.41–1.90)	0.03
LDL-C, mmol/L	3.64 (3.11–4.32)	3.63 (3.14–4.49)	0.88
Triglycerides, mmol/L	1.15 (0.87–1.47)	1.28 (1.00–1.67)	0.01
Fasting blood glucose, mmol/L	4.95 (4.57–5.26)	5.21 (4.48–5.47)	0.02
hs-CRP, mg/L	2.22 (1.10–4.22)	2.31 (1.14–3.93)	0.74
Fibrinogen, g/L	3.35 (3.00–3.78)	3.42 (3.00–3.86)	0.95
D-Dimers, ng/mL	609 (399–883)	816 (565–1,164)	0.04
Thrombin generation			
ETP, nmol/L/min	1,770 (1,601–1,961)	1,753 (1,563–1,898)	0.48
Peak height, nmol/L	337 (311–372)	340 (315–370)	0.64

Abbreviations: Bio = bioavailable; BMI = body mass index; E2 = estradiol; ETP = endogenous thrombin potential; GM = geometric mean; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MMSE = Mini-Mental State Examination; T = testosterone; WHR = waist-to-hip ratio.

[a] The *p* value from Cox model adjusted for age.

[b] Level of total cholesterol ≥6.20 mmol/L^{-1} or medication.

[c] Systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or medication.

[d] Fasting glycemia >7 mmol/L^{-1} or medication.

[e] Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale with a cutoff of >16.

Table 2 HRs (95% CIs) for all-cause dementia and AD associated with SSH levels in quartiles

	All-cause dementia (n = 132)					AD (n = 90)				
	No. of events	Model 1, HR (95% CI)	*p*	Model 2, HR (95% CI)	*p*	No. of events	Model 1, HR (95% CI)	*p*	Model 2, HR (95% CI)	*p*
Total-E2, pg/mL										
Q1: E2 ≤3.49	41	1.66 (0.91–3.02)	0.10	2.20 (1.07–4.52)	0.03	28	1.69 (0.83–3.43)	0.15	2.07 (0.89–4.75)	0.09
Q2: 3.49–5.30	26	1.19 (0.62–2.26)	0.61	1.46 (0.68–3.15)	0.33	18	1.22 (0.57–2.61)	0.62	1.37 (0.57–3.35)	0.48
Q3: 5.30–8.00	25	1 (reference)				17	1 (reference)		1 (reference)	
Q4: E2 >8.00	40	1.84 (0.99–3.39)	0.05	2.43 (1.15–5.20)	0.02	27	1.87 (0.90–3.91)	0.09	2.38 (0.99–5.75)	0.05
***p* for J-shaped**			0.03		0.006			0.03		0.01
Bio-E2, pg/mL										
Q1: Bio-E2 ≤2.29	44	1.62 (0.91–2.89)	0.10	1.95 (0.99–3.89)	0.05	30	1.48 (0.75–2.90)	0.25	1.66 (0.74–3.70)	0.22
Q2: 2.29–3.60	24	0.97 (0.51–1.87)	0.93	0.98 (0.42–2.27)	0.96	16	0.83 (0.38–1.81)	0.64	0.77 (0.30–2.03)	0.61
Q3: 3.60–5.60	25	1 (reference)		1 (reference)		19	1 (reference)		1 (reference)	
Q4: Bio-E2 >5.60	39	1.79 (0.97–3.30)	0.06	2.45 (1.19–5.05)	0.01	25	1.61 (0.78–3.30)	0.20	2.18 (0.94–5.00)	0.07
***p* for J-shaped**			0.04		0.005			0.10		0.01
Total-T, ng/mL										
Q1: total-T ≤0.21	42	1.22 (0.71–2.10)	0.475	1.33 (0.69–2.58)	0.393	25	1.06 (0.55–2.04)	0.866	1.15 (0.51–2.61)	0.729
Q2: 0.21–0.32	25	0.72 (0.40–1.30)	0.28	1.07 (0.55–2.07)	0.83	16	0.69 (0.34–1.39)	0.29	1.05 (0.47–2.33)	0.90
Q3: 0.32–0.45	27	0.67 (0.37–1.21)	0.18	1.02 (0.52–1.97)	0.95	23	0.81 (0.41–1.58)	0.53	1.52 (0.71–3.28)	0.28
Q4: Total-T >0.45	27	1 (reference)		1 (reference)		26	1 (reference)		1 (reference)	
***p* for trend**			0.45		0.86			0.98		0.89

Abbreviations: AD = Alzheimer disease; Bio = bioavailable; CI = confidence interval; E2 = estradiol; HR = hazard ratio; Q = quartile; SSH = steroid sex hormone; T = testosterone.
Model 1: adjusted for age and center; model 2: model 1 + education, *APOE* ε4, depressive symptoms, waist-to-hip ratio, Mini-Mental State Examination score at baseline, hypercholesterolemia, and history of myocardial infarction and stroke.

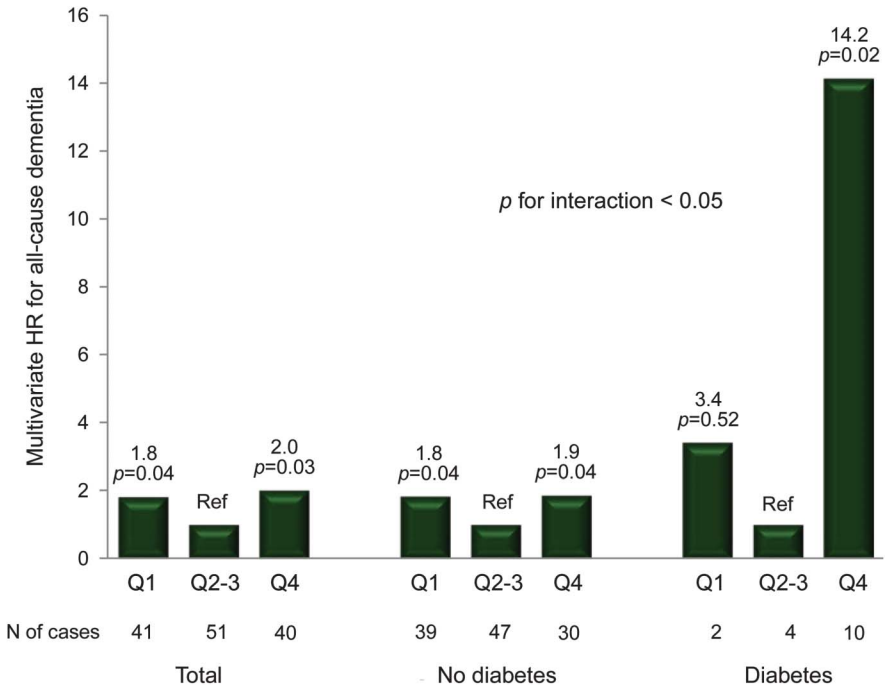
Sensitivity analysis. Excluding women with prevalent or incident coronary heart disease (n = 74 and n = 12, respectively) or stroke (n = 30 and n = 11, respectively) did not modify the association of SSHs with dementia. Interestingly, when women presenting with cognitive impairment at baseline (n = 98) or developing dementia within the first 2 years of follow-up (n = 67) were excluded, the association between low E2 and dementia was no longer increased (HR = 1.17 [0.45–3.00], p = 0.66), while risk of dementia remained significantly higher for women having a high total-E2 value (HR = 2.83 [1.01–8.33], p = 0.04) (figure 3).

DISCUSSION Using data from a large French prospective cohort study, we found that a higher level of endogenous E2 predicted incident all-cause dementia, independently of traditional dementia risk factors as well as inflammatory and blood coagulation markers. A low level of E2 also conferred a higher risk of dementia; however, sensitivity analyses suggested that reverse causation might have driven this finding. Finally, the risk associated with higher E2 levels was stronger in women with diabetes.

Even though surprising, given the expected neuroprotective effects of E2, more and more evidence suggests an association between high endogenous E2 and dementia in postmenopausal women. In the Rotterdam Study, higher total-E2 levels were associated with a 2-fold increased risk of incident dementia in more than 3,000 postmenopausal women.[6] However, unlike in our study, this association was confined to vascular dementia while no significant association was found for incident AD. In a smaller cohort of 433 Italian postmenopausal women, a higher E2 level also predicted the 4-year incidence of dementia.[7] This association pertained to both AD and vascular dementia, although not significant for the latter, probably because of a lack of statistical power. Of note, a high E2 level also predicted the 4-year cognitive decline in postmenopausal women from the Rancho Bernardo Study,[3] although other longitudinal studies reported either null[33,34] or negative[4,5] associations between E2 and cognitive performance. Overall, our results are compatible with data from clinical trials indicating null or deleterious impact of hormone therapy on cognitive function and dementia.[1]

We detected a significant interaction of increased E2 levels with diabetes on risk of dementia. One previous study investigated the interaction of high E2 levels with central adiposity, cholesterol levels, triglycerides,

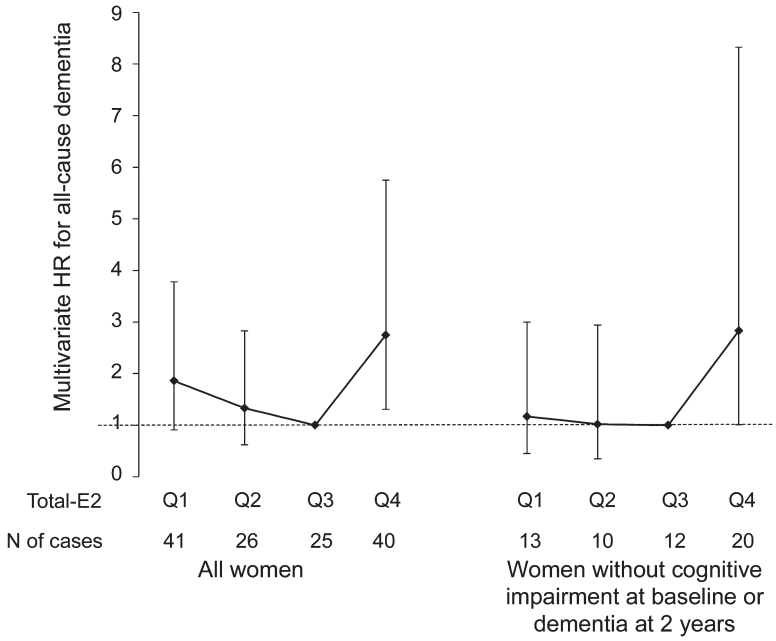
Figure 2 Total-E2 and all-cause dementia risk by diabetes status



Multivariate HRs for all-cause dementia associated with total-E2 levels in quartiles (Q2 and Q3 taken as the reference), by diabetes status. E2 = estradiol; HR = hazard ratio.

blood pressure, and glycemia on 4-year decline in memory tests.[3] In this study, in which increased E2 and estrone levels were associated with a 4-year decline in verbal fluency, no significant interaction of E2 with any cardiovascular risk factors was detected. Elevated E2 or estrone has been associated with a deleterious lipid profile[12] and diabetes[11,35,36] in other study samples. Taking into account triglycerides or HDL-C did not modify our results. However, we identified that women presenting with both diabetes and a high E2 level had a more than 14-fold increased risk of dementia. Diabetes is a major dementia risk factor, and various mechanisms are thought to explain this relationship.[16] Most importantly, brain infarcts, microvascular pathologies, inflammation, and alteration in glucose, insulin, and amyloid metabolism may underlie the increased dementia risk in subjects with diabetes. Given that high E2 is also associated with a range of mechanisms involved in atherothrombosis such as enhanced inflammation and blood coagulation,[13,14] it is plausible that higher endogenous E2 together with diabetes promote a set of unfavorable vascular processes to increase risk of dementia in postmenopausal women. Of note, considering inflammatory and hemostatic markers only slightly attenuated the association of high E2 with dementia and did not preclude the interaction of E2 with diabetes.

Figure 3 Total-E2 and all-cause dementia risk by severity of baseline cognitive status



Multivariate HRs for all-cause dementia associated with total-E2 levels in quartiles (Q3 taken as the reference) in all women and in women without cognitive impairment at baseline or dementia at 2 years. E2 = estradiol; HR = hazard ratio.

Other mechanisms may drive the association of higher E2 levels with risk of dementia. Conversion of testosterone to E2 by aromatase in adipose tissue is the main source of plasma E2 in postmenopausal women. The impact of high E2 on dementia could thus be driven by high fat mass or testosterone levels. In our study, neither adjustment for BMI nor for WHR modified our results. However, even if BMI

and most importantly WHR may represent good markers of visceral adiposity,[37] they do not constitute direct measures of fat or lean body mass, so residual confounding may remain. Regarding testosterone, the risk of dementia associated with high E2 remained unchanged after adjustment for total-T. Overall, further research is needed to better understand the mediators of E2's deleterious impact on dementia.

Lower E2 also conferred a higher risk of dementia in our study. Low E2 levels could be a consequence of the subclinical dementia process. This phenomenon of reverse causality could in part explain our results and might also explain why many case-control studies found either null associations between E2 and dementia or reported lower mean E2 levels in women with AD. In fact, in our study, women presenting with cognitive impairment at baseline or developing dementia within the first 2 years of follow-up had significantly lower E2 levels compared with noncases (data not shown). Most importantly, once these subjects were excluded, risk of dementia was no longer increased in women with lower E2 levels. We acknowledge that in this analysis, confidence intervals were very large and our results may thus reflect a lack of statistical power. Another potential explanation for the discrepancy between the findings of case-control studies pertains to the difference in assays' sensitivity to measure E2 levels.[38]

The main strengths of our study are its prospective design and the large number of carefully recorded sociodemographic and medical characteristics that could be accounted for in multivariate analyses. The quality of validation of dementia cases is also an important strength because it allowed the investigation of endogenous E2 levels in relation to dementia outcome, which has been less studied than cognitive decline. Some limitations need to be addressed. First, SSHs were measured using direct radioimmunoassay while radioimmunoassay after a step of purification or mass chromatography/mass spectrometry–based methods would have been more accurate, specifically to detect very low levels of plasma E2.[39] Imprecision in E2 levels using this kind of assay may have led to attenuation of the risk associated with E2 but was less likely to detect false associations. Second, the low number of incident dementia cases may have reduced our statistical power to detect significant associations of SSHs with DVC if they existed. Finally, although we ran-out sensitivity analyses in that sense, we cannot exclude that higher E2 levels may not actually be a risk factor for dementia but the consequence of the disease that may start more than 10 years before the appearance of the symptoms. A longer follow-up would allow clarification on this matter.

Our results provide further evidence that increased plasma E2 levels may be a risk factor for dementia in older postmenopausal women. Furthermore, women with high E2 levels and diabetes may represent a group at very high risk of dementia. Given the biological plausibility of this interaction and the expected increase in the number of elderly people with diabetes and/or dementia, more investigation on this topic should be urgently conducted.

AUTHOR CONTRIBUTIONS

J.-F.D. and P.-Y.S. conceived and designed the study. L.C. and P.-Y.S. performed statistical analysis, interpreted the data, and drafted the manuscript. A. G.-M. made the hormone determinations. J.-F.D., O.R., S.B.-T., A.G.-M., and M.-L.A. revised the manuscript. All authors approved the version to be published.

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DISCLOSURE

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